Relative Impact of Major Wine Polysaccharides on the Performances of an Organic Microfiltration Membrane

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Several main wine polysaccharides, isolated from a red wine and characterized in terms of composition and structural organization, have been microfiltered separately in a colloid-free synthetic wine to determine their respective incidence on the permeation fluxes of an hydrophilic organic microfiltration membrane. Most wine polysaccharides induced a significant decrease of the permeation flux density. The permeation flux decline were concentration-dependent and differed according to the nature and the composition of the polysaccharide tested. The hydrodynamic volume seemed to play a major role in determining the reduction of membrane permeability. Considering the mean amounts of the different polysaccharides in wines, it appeared that yeast mannoproteins displayed the strongest incidence on wine filtrability for the considered membrane. However, microfiltration experiments performed with a mixture of all polysaccharides tested pointed out that the pectic polysaccharides had a protective effect against the flux decrease induced by mannoproteins. The flux decline related to polysaccharides during wine microfiltration appears then to depend more on the respective amounts of the different polysaccharides than on the total polysaccharide content.

KEY WORDS: microfiltration, hydrophilic membrane, wine, polysaccharides, mannoproteins, arabinogalactans, rhamnogalacturonans, pectic polysaccharides

Cross-flow microfiltration has gained strong interest in the recent past years in enology [1-6,11,14,15,21,25,31]. One of its most promising potentialities is to allow the one-step clarification and sterilization of wines, which largely simplify the wine treatment lines. The first studies performed to evaluate cross-flow microfiltration performances pointed out the setting of a strong membrane fouling within the very first minutes of wine processing. This led to both dramatic flux declines and retentions of macromolecular colloids. Thereby to the efforts of scientists and industrials toward the selection of adequate membranes and operating conditions, macromolecule retentions have been lowered to become equivalent to those observed with usual filtration treatments [13,28]. However, permeation flux densities remain low and still alter the economic profitability of the cross-flow microfiltration process.

Previous studies have pointed out the incidence of wine polysaccharides on the performance of various microfiltration membranes, and demonstrated that their negative effect on permeation flow rates depended more on their composition and structure than on their concentrations in wines [1,2,3,30,31]. However, their exact behavior needs further elucidation. Numerous studies describe the composition and structure of the main wine polysaccharides [2,8,9,10,17,18, 19,20,23,24,27,29]. The aim of the present work was to investigate their separate capacities to foul an organic microfiltration membrane. This is required *(i)* to improve the knowledge of the role played by these macromolecules in flux reduction and *(ii)* to establish new technologies (enzymic degradation and/or new microfiltration membranes) to reduce their negative effect on microfiltration. To that purpose, several main wine polysaccharides have been isolated and purified from a red wine, and their respective impact on fluxes assayed in a synthetic wine.

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Manuscript submitted for publication 8 April 1998; revised 4 August 1998.

Materials and Methods

Wine sample and fractionation of polysaccharides. ***Polysaccharide purification:*** Polysaccharides were purified from a red wine prepared from mature grapes of Carignan noir harvested in 1991 at the INRA Pech-Rouge Enology Experimental Station (INRA, Gruissan, France). Total wine colloids were recovered by acidic-ethanol precipitation as reported [3]. Three successive chromatographic steps were then necessary to achieve the complete fractionation of wine polysaccharides [12,18,19,26,29]. They were first separated as a function of their negative charge densities by two successive steps of anion-exchange chromatography on a DEAE-Macroprep column (5 × 80 cm; Bio-Rad, USA) equilibrated at 20 mL/min in 50 m*M* acetate buffer pH 4.6 next 5.6, elution being performed by applying step-wise NaCl-concentration gradients. Polysaccharides were then fractionated as a function of their hydrodynamic volume by size-exclusion chromatography on a Sephacryl S-400-HR column (5 × 80 cm; Pharmacia, Sweden) equilibrated in 50 m*M* acetate buffer pH 5.0, containing 50 m*M* NaCl. Yeast mannans were finally separated from grape polysaccharides by affinity chromatography on Concanavalin A-Sepharose (5 × 15 cm, Pharmacia). Polysaccharides were recovered after extensive dialysis against water and freeze-dried.

Nature and composition of wine polysaccharidic fractions: Five fractions, representing the main wine polysaccharides and whose compositions are given in Table 1, have been used for the present study. Their homogeneity, estimated by their glycosyl-residue composition and size exclusion chromatography, was in all cases higher than 90%. These fractions were:

- Rhamnogalacturonan I (RG-I), whose composition is complex but dominated by rhamnose and uronic acid [16]. Its high charge density is related to its high galacturonic acid content and increases dramatically in the wine pH range (unpublished data).
- Rhamnogalacturonan II (RG-II), known as the most complex pectic polysaccharide [16,22], with the presence of several characteristic sugars and a high degree of linkage on a short homogalacturonan backbone. Its charge density is related to the presence of several acidic sugars [8,19] and increases dramatically between pH 2 and 5 [26].
- Two arabinogalactan-proteins (AGP) with increasing negative charge densities, described in a previous work as AGP0 and AGP3 [18]. They present all the characteristics of type II AGPs, but differ mainly by their uronic acid content (Table 1). Their negative charge densities are related to the dissociation of the carboxylic functions of their uronosyl groups and increase between pH 2 and 5 [26].
- A yeast-originating mannoprotein (MP), whose structural composition is characteristic of yeast cell wall mannoproteins with a composition dominated by mannose and a low protein content. Its net charge density, attributed to its phosphorus content, is pH independent at the wine pH range [26].

Microfiltration experiments. ***Model solutions:*** The fouling effect of each polysaccharide was assayed at different concentrations in a synthetic wine composed of: ethanol 12% v/v; glycerol, 7 g/L; K_2SO_4, 2.234 g/L; $MgCl_2 \cdot 6H_20$, 0.4 g/L; $CaCl_2 \cdot 2H_20$, 0.36 g/L; 1 *M* citrate buffer pH 3.5, 100 mL/L. Given the concentration of potassium in the model wine, citric acid was used instead of tartaric acid to avoid potassium hydrogen tartrate precipitation in the polarization concentration layer, potassium hydrogen tartrate being highly instable in a colloid-free synthetic solution. The concentrations tested were chosen on the basis of the concentration range of each polysaccharide in wine [20] and were: RG-I, 20 to 100 mg/L; RG-II, 50 to 100 mg/L; AGP0, 100 to 200 mg/L; AGP3, 30 to 100 mg/L; MP, 20 to 200 mg/L. The membrane fouling caused by a mixture of all these polysaccharides was studied with their respective average concentration in red wines, i.e. RG-I, 20 mg/L; RG-II, 100 mg/L; AGP0, 150 mg/L; AGP3, 30 mg/L; MP, 150 mg/L. The model wines containing the polysaccharides were prepared one hour prior to the filtration experiment.

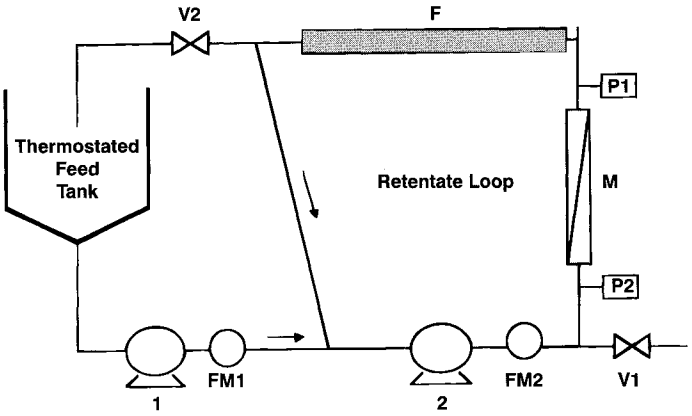

Fig. 1. Schematic view of the experimental microfiltration device. M, microfiltration membrane; 1 and 2, volumetric pumps; V1 and V2, valves; P1 and P2, pressure gauges; FM1 and FM2, flow-meters; F, thermostatically controlled freezing system.

Cross-flow microfiltration: Microfiltration experiments were performed with a laboratory-sized plant (Fig. 1) equipped with an organic modified polyethersulfone membrane (M202) provided by X-flow (The Netherlands). This capillary membrane had a maximum pore size of 0.2 μm, an average pore size of 0.08 μm, and internal and external diameters of 1.5 and 2.5 mm. The filtration area was 20 cm^2, and new membrane material was used for each experiment. The operating conditions were: filtered volume 750 mL; transmembrane pressure, 140 kPa; tangential flow velocity 4 m/sec; temperature 20°C; constant volumetric concentration factor. The permeation flux density was determined by weighing. Each result represent the mean of at least two separate experiments (5 experiments with the synthetic wine).

Results

Microfiltration profiles: Model solutions of each polysaccharide fraction were microfiltered in comparison with the colloid-free synthetic wine (Fig. 2a - e). Various concentrations were tested and average flux densities over the whole experiment, calculated from permeation curves, are reported in Figure 3. The permeation flow density of the colloid-free synthetic wine stabilized after 10 to 15 minutes of filtration. The model solutions of the various polysaccharides displayed different behaviors. The presence of RG-II had no detectable incidence on flux with regards to the colloid-free synthetic wine, whereas all other polysaccharides caused a reduction of the permeation flux density, to an extent depending on their nature and composition.

Mean flux densities observed for the model solutions of AGP0 were from 30 (100 mg/L) to 47% (200 mg/L) lower than the mean flux density of the colloid-free synthetic wine. Microfiltration curve profiles were similar, with a decrease of the flux during the initial 20 minutes of the experiment followed by a stabilization to a constant value. At a same concentration of 100 mg/L, the MP, AGP3, and RG-I fractions caused higher flux reductions, from 62% to 87%. The strongest drops in flux densities were induced by mannoproteins. With

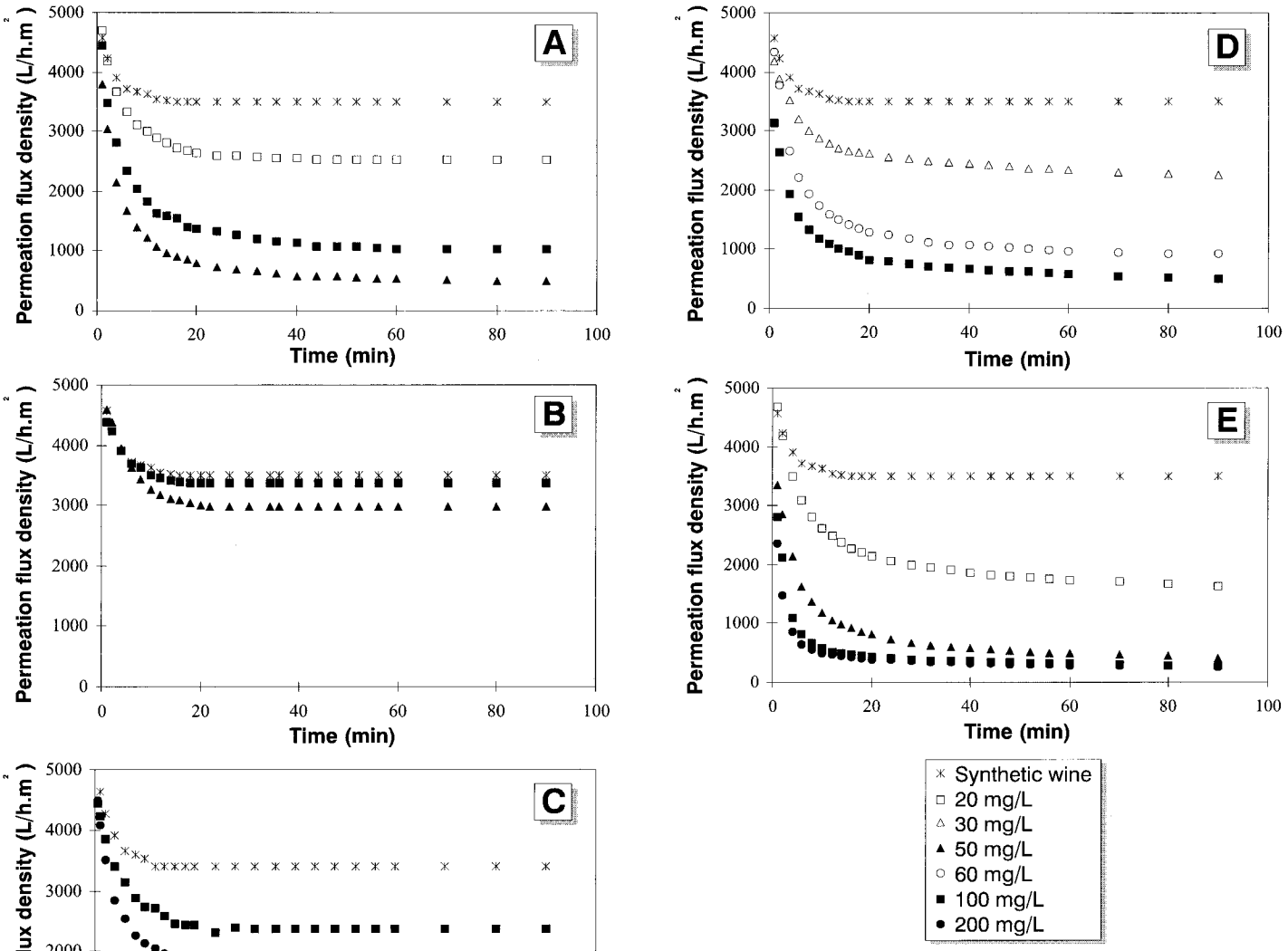

Fig. 2. Microfiltration profiles of the synthetic wine and of model solutions of polysaccharidic fractions. **A**: RG-I; **B**: RG-II; **C**: AGP0; **D**: AGP3; **E**: MP.

these polysaccharides, the initial rapid decrease of the permeation flux density was followed by a very progressive decline. In the tested concentration ranges, flux reduction was concentration-dependent. A plateau value was observed for RG-I and MP for concentrations above 50 and 100 mg/L, respectively.

The incidence of a mixture of all polysaccharides tested was also assayed at their average concentrations in red wines [20]. The permeation flow curve obtained with the whole mixture was compared to that of the corresponding solutions of individual polysaccharides at a same concentration (Fig. 4). Surprisingly, the fouling effect of the whole mixture appeared to be not additive, but cooperative. The flux density of the mixture was between that of pectic polysaccharides and mannoproteins: the initial flux decline was less pronounced than with the mannoproteins alone and the flux density remained slightly higher over the whole experiment. This nonadditive effect of the combination of wine polysaccharides on fluxes is also underlined by the comparison of the mean permeation flux densities obtained with individual polysaccharides and the mixture (Fig. 3).

Discussion

The main wine polysaccharide fractions have been characterized in our laboratory in terms of composition and structural organization [2,8,9,17,18,19,20, 23,24,29], and some of their physicochemical properties have been determined [26]. The aim of this work was to study their separate capacity to foul an hydrophilic capillary microfiltration membrane and to discuss this capacity with respect to *(i)* their known structural and/or physicochemical characteristics and *(ii)* their respective amounts in wines as a function of winemaking technologies. Most wine polysaccharides tested induced a significant decrease of the permeation flux density, but the extent of this decrease depended on their na-

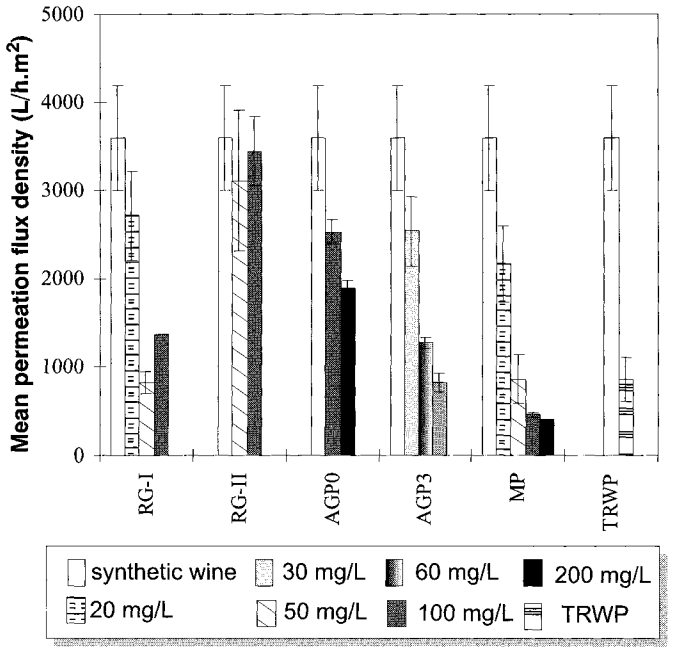

Fig. 3. Mean fluxes calculated from permeation curves for each of the tested polysaccharide fraction and each concentration. TRWP: mixture of polysaccharides.

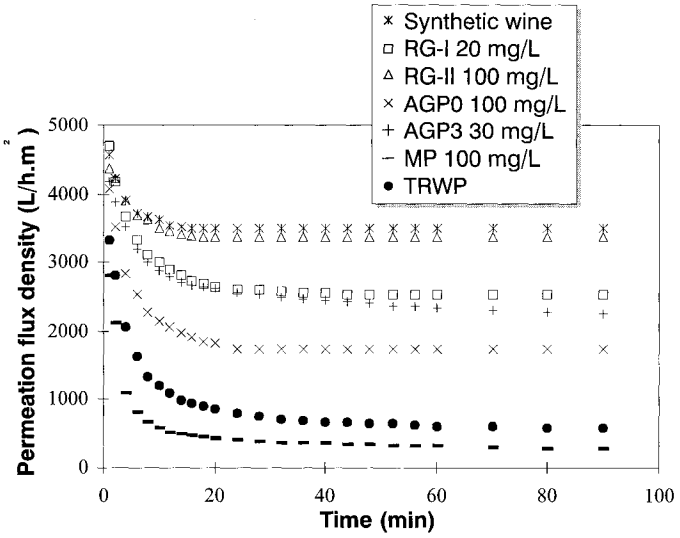

Fig. 4. Microfiltration curve profile of the mixture of polysaccharides (TRWP) , in comparison with that of each of the separate fraction at its mean concentration in the blend.

ture and composition. Such a negative influence of wine macromolecules on cross-flow microfiltration performances had been previously reported on both polysulfone [30] and alumina [1,2,3] membranes.

Wine polysaccharides could be ranked from the present experiments according to their increasing effect on flux as follows: RG-II < AGP0 < RG-I ∪ AGP3 < MP. Rhamnogalacturonan II, a small acidic pectic polysaccharide (average molecular weight 10 000), did not affect significantly the permeation flow rate. This result differed from a previous study [2,3], where a small acidic polysaccharide fraction was shown to be involved in membrane fouling during the microfiltration of a red wine. Although RG-II had not been identified as a wine component at the time of this previous experiment, it is now clear that this low molecular weight acidic fraction was mainly composed of RG-II. In this previous study, the microporous material was an alumina membrane and the part played by the RG-II-containing fraction in flux reduction was attributed to attractive electrostatic or ionic interactions between positively charged alumina and negatively charged acidic RG-II at the wine pH (Table 1). These interactions induced a strong adsorption of RG-II on the membrane surface and an internal fouling. The membrane studied in the present investigation, due to the functional groups of its constitutive polymers, is expected to be negatively charged so that an attractive interaction with uronic acid-containing polysaccharides is most unlikely.

The other polysaccharides tested, whose average molecular weights are much higher, exhibited significant effects on flux reduction. Type II arabinogalactan-proteins and RG-I form homogeneous fractions with respective average molecular weights of 165 000 for AGP0, 240 000 for AGP3 [17,18], and 50 000 to 70 000 for RG-I [Doco T., unpublished data, 1997]. The MP fraction is polydisperse, with a broad molecular weight range from 30 000 to 400 000. However, the incidence of wine polysaccharides on flux seems to be related to their hydrodynamic volume and radius of gyration rather than to their average molecular weight. For instance, if the respective effects of compact polysaccharides with a high degree of ramification as RG-II, MP, AGP0, and AGP3 could be directly correlated with their respective molecular weights, RG-I, which has an extended helix-shape conformation [7], diverged from the correlation. No apparent correlation between flux reduction and negative charge densities can be deduced from the comparison of the effect of the different fractions tested. RG-I and RG-II show the highest negative charge densities among wine polysaccharides [26] but displayed totally different behaviors.

Cross-flow microfiltration performances strongly depend on the nature of the processed wine, but the relationship between the wine filtrability and its composition still remains unclear. The wine composition in polysaccharides is one of the parameters which influences its filtrability [1,5]. The usual concentration ranges of each of the polysaccharide tested in white and red wines are reported in Table 2. The major ubiquitous polysaccharides are arabinogalactan-proteins and mannoproteins; the respective amounts of pectic polysaccharides may depend on the prefermentation practices, whose incidence is still poorly understood, and on the importance of the maceration intensity [20].

The microfiltration experiments performed with model solutions indicated that yeast mannoproteins may have the strongest incidence on wine filtrability. Their incidence on flux is concentration-dependent, but a plateau value is reached at wine concentration range. Thus, the differences observed between wines during

Table 1. Chemical composition and physico-chemical characteristics of the studied polysaccharidic fractions.

	RG-I	RG-II	AGP0	AGP3	MP
proteins (a)	1.6	0.6	3.6	2.4	6.2
uronic acids (a)	42.4	39.0	2.7	12.4	
neutral sugars (a)	44.2	24.9	79.5	77.0	92.7
phosphorus (a)					0.1
negative charge density (meq/g)	2.44	1.82	0.05	1.40	0.08
molecular weigth distribution	50 000-70 000	9 000-10 000	150 000-170 000	220 000-240 000	30 000-400 000
Glycosyl residues (b)					
rhamnose	37.9	16.9	1.1		
arabinose	8.3	9.7	40.5		
xylose	2.9			7.1	
mannose	1.7		0.5	43.2	0.5
galactose	7.9	5.0	53.8		
glucose	0.7		1.0	1.0	93.6
2-*O*-methyl-fucose	0.5	4.2		39.8	1.5
fucose	3.4	3.6		0.9	4.4
2-*O*-methyl-xylose		3.1			
apiose		7.2			
glucuronic acid	2.9	3.4	3.1		
galacturonic acid	33.8	37.2			
aceric acid		2.2		6.1	
Dha (c)		2.5		1.9	
Kdo (c)		5.0			

(a) % dry matter; (b) Mol %; (c) Kdo (3-Deoxy-D-*manno*-2-octulosonic acid), Dha (3-Deoxy-D-*lyxo*-2-heptulosaric acid).

microfiltration cannot be directly attributed to their respective amounts in mannoproteins. At their mean concentrations in wines, pectic polysaccharides did not significantly affect the fluxes when compared to mannoproteins (Fig. 4).

When considering the microfiltration experiments performed with the mixture of all the polysaccharides, it becomes obvious that the previous conclusions have to be shadowed: there was no simple additive effect of the polysaccharides on fluxes. Microfiltration curve profiles pointed out that the pectic polysaccharides can have a protective action toward the loss of permeability induced by mannoproteins. As a consequence, it can be assumed that flux decline related to polysaccharides during wine microfiltration may depend more on the balance between the different polysaccharidic classes than on the global polysaccharide concentration. This is in accordance with the previous observations performed with different wines and different microfiltration membranes.

Table 2. Concentration range (in mg/L) of the main wine polysaccharide classes in wines [23].

	white wines	red wines
RG-I	10 - 20	20 - 50
RG-II	20 - 50	50 - 150
AGP0	50 - 100	100 - 150
AGP3	10 - 30	30 - 50
MP	100 - 150	100 - 150

Conclusions

Most wine polysaccharides induced in model solution a decrease of the permeation fluxes of an hydrophilic organic microfiltration membrane. The importance of the permeation flux decline was shown to be concentration-dependent. It also depended on the average hydrodynamic volume of each polysaccharide. Indeed, a relationship could be established with globular polysaccharides (RG-II, AGP0, AGP3, and MP) between their average molecular weight and their negative incidence on fluxes. RG-I diverged from this correlation but has an extended helix-shape conformation.

At their mean concentrations in wines, mannoproteins are the polysaccharides which exhibited the strongest effect on fluxes. This effect was reduced in the presence of the pectic polysaccharides, suggesting that the membrane performances will be dependent on the balance between yeast and grape providing carbohydrate polymers.

Literature Cited

1. Belleville, M. P., J. M. Brillouet, *et al.* Polysaccharide effects on cross-flow microfiltration of two red wines with a microporous alumina membrane. J. Food Sci. 55:1598-1602 (1990).

2. Belleville, M. P., J. M. Brillouet, *et al.* Differential role of red wine colloids in the fouling of a cross-flow microfiltration alumina membrane. Vitic. Enol. Sci. 46:100-107 (1991).

3. Belleville, M. P., J. M. Brillouet, *et al.* Fouling colloids during microporous alumina membrane filtration of wine. J. Food Sci. 57: 396-400 (1992).

4. Brillouet, J. M., M. Moutounet and J. L. Escudier. Fate of yeast and

grape pectic polysaccharides of a young red wine in the cross-flow microfiltration process. Vitis 28:49-63 (1989).

5. Cameira dos Santos, P. J., V. Cheynier, *et al.* Cross flow microfiltration of wines with polymeric membranes: the influence of wine making technology and backpulse on the fouling process. Prehrambeno-Tecnol. Biotecnol. Rev. 32:77-83 (1994).

6. Cameira Ferreira dos Santos, P.J. Colmatage en microfiltration tangentielle: mise en évidence d'interactions entre les polysaccharides et les polyphénols d'un vin et des membranes polymériques. Thesis, University of Montpellier II, Ecole Nationale Supérieure Agronomique de Montpellier (1995).

7. Cros, S., C. Garnier, *et al.* Solution conformations of pectic polysaccharides: determination of chain characteristics by small angle neutron scattering, viscometry and molecular modeling. Biopolymers 39:339-352 (1996).

8. Doco, T. and J. M. Brillouet. Isolation and characterization of a rhamnogalacturonan II from red wine. Carbohydr. Res. 243:333-343 (1993).

9. Doco, T., N. Quellec, *et al.* Evolution of polysaccharides during the aging of Carignan noir red wines. Am. J. Enol. Vitic. 50:25-32 (1999).

10. Dubourdieu, D., D. Hadjinicolaou, and P. Ribéreau-Gayon. Structure of the exocellular ß-D-glucan from *Botrytis cinerea*. Carbohydr. Res. 93:294-299 (1981).

11. Feuillat, M., D. Peyron, and J. L. Berger. Influence de la microfiltration tangentielle des vins sur leur composition physico-chimique et leurs caractères sensoriels - Application aux vins de Bourgogne. Bull. OIV 60:227-244 (1987).

12. Gerbaud, V., N. Gabas, *et al.* Influence of wine polysaccharides and polyphenols on the cristallization of potassium hydrogen tartrate. J. Int. Sci. Vigne Vin 31:65-83 (1997).

13. Humbert, C. La microfiltration tangentielle des vins sur membrane Kerasep. Rev.Œnol. 69:25-26 (1993).

14. Lüdemann, A. Wine clarification with a cross-flow microfiltration system. Am. J. Enol. Vitic. 38:228-235 (1987).

15. Moutounet, M., J. L. Escudier, and E. Montaigne. Application des techniques à membrane aux produits de la vigne - réflexions sur leurs perspectives de développement. Rev. Fr. Oenol. 119:31-36 (1989).

16. O'Neill, M., P. Albersheim, and A. Darvill. The pectic polysaccharides of primary cell walls. Meth. Plant Biochem. 2:415-441 (1990).

17. Pellerin, P., E. Waters, and J. M. Brillouet. Characterization of two arabinogalactan-proteins from red wine. Carbohydr. Polym. 22:187-192 (1993)

18. Pellerin, P., S. Vidal, *et al.* Characterization of five type II arabinogalactan-protein fractions from red wines of increasing uronic acid content. Carbohydr. Res. 277:135-143 (1995).

19. Pellerin, P., T. Doco, *et al.* Structural characterization of red wine rhamnogalacturonan II. Carbohydr. Res.: 290, 183-197 (1996).

20. Pellerin, P., and Cabanis J.C. Les glucides. *In:* Eléments d'Œnologie. Flanzy C. (Ed.). pp 40-92. Lavoisier Tec et Doc, Paris. In Press (1998).

21. Peri, C., M. Riva, and P. Decio. Cross-flow membrane filtration of wines: comparison of performance of ultrafiltration, microfiltration and intermediate cutt-off membranes. Am. J. Enol. Vitic. 39:162-168 (1988).

22. Puvanesarajah, V., A. G. Darvill, and P. Albersheim. Structural characterization of two oligosaccharide fragments formed by selective cleavage of Rhamnogalacturonan. II: Evidence for the anomeric configuration and attachment sites of apiose and 3-deoxy-2-heptulosaric acid. Carbohydr. Res. 218:211-222 (1991).

23. Saulnier, L., J. M. Brilloue,t and J.P. Joseleau. Structural studies of pectic substances from the pulp of grape berries. Carbohydr. Res. 182:63-78 (1989).

24. Saulnier, L., J. M. Brillouet, *et al.* Structural investigation of an arabinigalactan from grape juice. Carbohydr. Polym. 127:59-73 (1992).

25. Serrano, M., B. Potens, and P. Ribéreau-Gayon. Etude de différentes membranes de microfiltration tangentielle. Comparaison avec la filtration sur précouche de diatomées. J. Int. Sci. Vigne Vin 26:97-116.

26. Vernhet, A., P. Pellerin, *et al.* Charge properties of some grape and wine polysaccharidic and polyphenolic fractions. Am. J. Enol. Vitic. 41:25-30 (1996).

27. Villetaz J. C., R. Amado, and H. Neukom. Structural investigation of an arabinan from grape juice. Carbohydr. Polym. 1:101-105 (1981).

28. Vogt Kolumban. Le développement Filtrox en filtration tangentielle. Rev. Œnol. 69:21-24

29. Waters E.J., P. Pellerin and J.M. Brillouet. A Saccharomyces mannoprotein that protects wine from protein hazes. Carbohydr. Polym. 23: 185-91 (1993).

30. Wörner K., R. Weinand, H. Dietrich. Investigations on the membrane fouling during the cross flow filtration of wines. Vitic. Enol. Sci. 49:170-9 (1994).

31. Wucherpfennig, K., H. Dietrich, and R. Fauth. Über den einflub von polysacchariden auf die klärung und filtrierfähigkeit von weinen unter besonderer Berücksichtigung des Botrytisglucans. Deut. Lebensmittel-Rundschau 80:38-44 (1984).